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Docket No.: P02186US0

CLEAN COPY OF ALL PENDING CLAIMS AS OF MAY 18, 2001

camphor monooxygenase cytochrom P450

subspecies 3: mutants of P450CAM or homologues

1. (Amended) Process for oxidising a substrate which is an acyclic or cyclic terpene, or a cycloalkene or a substituted derivative thereof, and which process comprises: oxidising said substrate with a mutant haem-containing enzyme, the mutant comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain.

using Beef Carmichael

genus

2. Process according to claim 1 in which the enzyme is a mutant of $P450_{cam}$ or $P450_{BM-3}$, or a mutant of a naturally occurring homologue of either of these enzymes.

3. (Amended) Process according to claim 2 in which the enzyme is one in which amino acid 47 or 51 or 47 and 51 of $P450_{BM-3}$, or amino acid 96 of $P450_{cam}$, or the equivalent amino acid in a said homologue, have been changed to an amino acid with a less polar side-chain.

12.1st 1,2,4

12.2nd 3 + 5

4. Process according to claim 1 in which there are one or more other amino acid substitutions in the active site.

5. Process according to claim 1 in which the enzyme is (i) $P450_{cam}$ and comprises one or more of the following mutations: F87W, F87I, F87L, T185L, T185F, V247A, V247L or F87A-I395F; or (ii) $P450_{BM-3}$ and comprises the mutation R47L-Y51F.

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6. (Amended) The enzyme as defined in claim 4 excluding mutants of $P450_{cam}$ which only have the mutations F87A-Y96G-F193A, F87A-Y96G-F193A-C334A, or T101M-T185F-V247M.

term is present Chloroform

7. A polynucleotide which comprises a sequence which encodes an enzyme as defined in claim 6.

8. (Amended) A cell which expresses:

(i) a mutant haem-containing enzyme comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain which in its naturally occurring form has an electron transfer reductase domain, or

(ii) (a) a mutant haem-containing enzyme comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain,

(b) an electron transfer reductase, and

(c) an electron transfer redoxin; or

(iii) (a) (1) $P450_{cam,}$ or a fragment thereof; or

(2) a naturally occurring homologue of $P450_{cam}$ or a fragment thereof; or

(3) a mutant $P450_{cam}$, or a mutant of a naturally occurring homologue of $P450_{cam}$ comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain; or

(4) a $P450_{cam}$ which has at least 70% amino acid homology with (1), (2) or (3) and optionally in which amino acid 96 has been changed to an amino acid with a less polar side-chain; and

(b) an electron transfer reductase; and

(c) an electron transfer redoxin, or

(iv) (a) (1) $P450_{BM-3}$, or a fragment thereof; or

(2) a naturally occurring homologue of $P450_{BM-3}$ or a fragment thereof; or

(3) a mutant $P450_{BM-3}$, or a mutant of a naturally occurring homologue of $P450_{BM-3}$ comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain

excluding an *E. Coli* DH5α cell in which the only mutants of $P450_{cam}$ which are expressed are amongst the following:

H_2N-$P450_{cam}$-TDGTSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H_2N-$P450_{cam}$–TDGTRPGPGPGPGPSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H_2N-$P450_{cam}$–TDGTRPGPGPGPGPGPGPSST-putidaredoxin reductase-TDGASSS putidaredoxin-COOH,

H_2N-450_{cam}–putidaredoxin reductase-TDGASSS-putidaredoxin-PLEL-$P450_{cam}$-COOH.

9. (Amended) The cell according to claim 8 in which (a), (b) and (c) or (b) and (c) are expressed together in the same fusion protein.

10. (Amended) The cell according to claim 8 in which:

(b) is putidaredoxin reductase or a fragment thereof; and/or

(c) is putidaredoxin or a fragment thereof.

11. (Amended) Process for oxidising a substrate which is an acyclic or cyclic terpene, or a cycloalkene, or a substituted derivative thereof, and which process comprises oxidising said substrate with a mutant haem-containing enzyme, the mutant comprising a substitution of a first amino acid in an active site by an amino acid with a less polar side-chain, wherein the substrate is oxidised in a cell according to claim 8.

12. A process for making a library of mutants of $P450_{cam}$ or $P450_{BM-3}$, or mutants of a homologue of either of these enzymes comprising contacting a cell according to claim 8, or an *E.Coli* DH5α cell in which the only mutants of $P450_{cam}$ which are expressed are amongst the following:

H_2N-$P450_{cam}$-TDGTSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H_2N-$P450_{cam}$-TDGTRPGPGPGPGPSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H_2N-$P450_{cam}$-TDGTRPGPGPGPGPGPGPSST-putidaredoxin reductase-TDGASSS-putidaredoxin-COOH,

H_2N-putidaredoxin reductase-TDGASSS-putidaredoxin-PLEL-$P450_{cam}$-COOH;

with a mutagen and/or when the cell is a mutator cell culturing the cell in conditions in which mutants are produced.

13. (Amended) Process for selecting a mutant of $P450_{cam}$ or $P450_{BM-3}$, or a homologue thereof, for its ability to oxidise a substrate, which process comprises screening a group of said mutants for their oxidation effect on the substrate.

14. (Amended) Process according to claim 13 in which the mutant is additionally selected for its ability to oxidise the substrate to an oxidation product.

15. (Amended) Process for selecting a mutant of $P450_{cam}$ or $P450_{BM-3}$, or a homologue thereof, for its ability to oxidise a substrate, which process comprises screening a group of said mutants for their oxidation effect on the substrate in which the screening is carried out on the library made in a process according to claim 12.

16. (Amended) A process for producing a library of oxidation products comprising providing an acyclic or cyclic terpene, or a cycloalkene, or a substituted derivative thereof to a library made in a process according to claim 12 and allowing oxidation of the substrate.

17. (Amended) A method of treatment of a human or an animal comprising administering an oxidation product obtained by oxidising a substrate which is an acyclic or cyclic terpene, or a cycloalkene, or a substituted derivative thereof, and which process comprises oxidising said substrate with a mutant haem-containing enzyme, the mutant comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain, wherein optionally the enzyme is one which has been selected in a process according to claim 13.

18. (Amended) A pharmaceutical composition comprising an oxidation product obtained by oxidising a substrate which is an acyclic or a cyclic terpene, or a cycloalkene, or a substituted derivative thereof, and which process comprises oxidising said substrate with a mutant haem-containing enzyme, the mutant comprising a substitution of an amino acid in an active site by an amino acid with a less polar side-chain, wherein optionally the enzyme is one which has been selected in a process according to claim 13 and a pharmaceutically acceptable carrier or diluent.